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Common epitopes between mycobacterial and certain host tissue antigens

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SUMMARY

Monoclonal antibodies (MoAb) produced against a sonicated antigen extract of *Mycobacterium bovis* were examined for their ability to bind to 10 common tissue antigens. The binding patterns of MoAb MB2, MB3, MB7, MB11 and MB13 indicated that each recognized different epitopes. MoAb MB5 and MB17 failed to bind to any of the tissue antigens. MB3 reacted strongly with normal tissue from *M. bovis* infected guinea pigs and ferrets but there was no reaction with MB5 and MB17. These specificities were confirmed by reciprocal absorption experiments. It is concluded that *Mycobacterium* species contain epitopes that are also present in host tissue antigens.

Keywords tissue antigens mycobacteria

INTRODUCTION

Antigenic similarities between host tissue and bacterial components include those between blood group substances and bacterial polysaccharides (Drach, Reed & Williams, 1971), cardiac tissue and streptococcal polysaccharides (Nakhla & Glynn, 1967), kidney tissue and *Escherichia coli* lipopolysaccharides (Holmgren *et al.*, 1971; Drach *et al.*, 1971) and brain components and the capsular polysaccharides of *Neisseria meningitidis* group B and of *E. coli* K1 (Finne, Leinonen & Mäkelä, 1983). The biological significance of these cross reactions is not yet clear but their involvement in tissue damage has been suggested (Jann & Westphal, 1975; Avrameas *et al.*, 1983) and severe restrictions to the immune defence against bacteria containing K1 polysaccharide antigen have been demonstrated (Finne *et al.*, 1983).

Recently monoclonal antibodies (MoAb) have been produced against *Mycobacterium bovis* antigens (Morris, Thorns & Woolley, 1985) and some of these MoAb reacted with a wide range of mycobacterial species. Competitive antibody binding assays using serum from a range of apparently healthy animals indicated that these sera contained antibodies that bind to the same epitopes as those identified by our non-specific MoAb (unpublished data). This prompted us to examine MoAb for their ability to bind to host tissue proteins. We chose proteins (a) that are known to be well conserved during evolution, (b) that constitute genuine autoantigens and (c) that had been shown to react with natural antibodies (Avrameas *et al.*, 1983).

MATERIALS AND METHODS

MoAb. The production and partial characterization of the murine MoAb has been described (Morris *et al.*, 1985). Table 1 summarises the properties of the MoAb used. Hybridoma cell supernatants were used in all the experiments.

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Mouse antibody. Pooled serum from five BALB/c mice immunised with *M. bovis* and pooled serum from five control mice were used.

Antigens. M. bovis strain AN5 was grown on non-antigenic modified Sauton's medium (Stanford & Beck, 1968) and a sonicate prepared as described by Morris *et al.* (1985) and stored at −20°C. An *E. coli* surface antigen was prepared and stored at 4°C (Morris, Stevens & Sojka, 1977). Actin (bovine and rabbit muscle), albumin (bovine type V and egg type VI), collagen (human type VI), cytochrome C (horse heart type VI), DNA (type VIII from *E. coli*), fetuin (type IV), myoglobulin (dog muscle type V), myosin (rabbit muscle), thyroglobulin (bovine type I) and transferrin (human) were purchased from Sigma Chemical Company Ltd (London).

Binding assays. Indirect enzyme linked immunosorbent assays (ELISA) were performed to detect binding of MoAb to the antigens. Antigens were coated onto polyvinyl chloride microtitration plates (Falcon, Becton–Dickinson) at a concentration of 5 μg/well in 0·1 M carbonate–bicarbonate buffer, pH 9·5 for 2 h at 37°C, followed by overnight incubation at 4°C. Plates were then washed with phosphate-buffered saline (PBS) containing 0·05% Tween 20 and incubated with the MoAb (250 ng/well) overnight at 4°C. Plates were washed six times in PBS + 0·05% Tween 20 and an optimum dilution of goat anti-mouse IgG + IgA + IgM, peroxidase

Table 1. Properties of MoAb used in this paper

MoAb	Immunoglobulin class	Mycobacterial antigen used for production of MoAb	Specificity
MB2, MB3	IgMκ	BCG sonicate	Wide range of mycobacteria
MB7, MB11, MB13	IgMκ	*M. bovis* strain 117 sonicate	Wide range of mycobacteria
MB17	IgAκ	*M. bovis* strain 117 sonicate	Wide range of mycobacteria
MB5	IgG2aκ	*M. bovis* strain 117 sonicate	*M. bovis* only

conjugate (Cappel Laboratories, Dynatech Labs., UK) was added to the wells and incubated for 2 h at 37°C. After washing the reaction was detected using purified 5-amino-salicylic acid (Ellens & Gielkens, 1980). The results are expressed as the percentage of antibody binding to the test antigens relative to the binding in the high control in which normal mouse serum was used in place of antigen. All tests were performed in duplicate and the mean results recorded.

In another series of assays MoAb were pre-incubated (at a concentration that gave 75% binding to the homologous sonicated antigen) with various tissue antigens at 500 to 0·1 μg/ml overnight at 4°C. Indirect ELISA were then performed on the absorbed MoAb as described above.

Immunoperoxidase technique. Paraffin embedded pathological sections of mesenteric lymph nodes from normal and *M. bovis* infected ferrets and guinea pigs (Thorns, Morris & Little, 1982) were cut at 5 μm and stained using a modification of the protocol described previously (Bailey, Wells & Sheehan, 1985). After dewaxing, endogenous peroxidase was inhibited by the addition of 0·5% methanolic hydrogen peroxide. Sections were pre-incubated with freshly prepared trypsin and incubated with MoAb (500 ng/section) overnight at 4°C. After washing, an optimum dilution of peroxidase conjugated goat anti-mouse IgG + IgA + IgM antibody was added and the sections incubated for 2 h at 37°C after which the reaction was visualized by the addition of diaminobenzidine and hydrogen peroxide. Sections were then counterstained with Mayer's haemalum.

RESULTS

Binding of antibody to tissue antigens

Preliminary experiments showed that many of the MoAb bound to the blocking agent bovine serum albumin. The coating of the plates with antigen (5 μg/well) was shown to be sufficient to prevent direct binding of MoAb to plastic. Blocking agent was not used in the experiments reported.

The binding of MoAb to tissue antigens is shown in Table 2. MoAb MB17 and MB5 did not bind to any of the tissue antigens examined ($< 5\%$) whereas both bound to their homologous sonicated antigen ($> 40\%$). MoAb MB2, 3, 7, 11 & 13 each bound to a number of tissue antigens with the exception of collagen (Table 2). These MoAb could be differentiated on their binding patterns to a selected group of antigens (Table 3).

Pooled polyclonal serum from normal mice bound to all the antigens examined with the exception of collagen, but the binding with pooled serum from mice sensitised with *M. bovis* was considerably greater. This was most pronounced with transferrin, egg albumin and DNA (Table 4).

Table 2. Binding of MoAb to homologous and tissue antigens

	MoAb						
Antigens	MB2	MB3	MB7	MB11	MB13	MB17	MB5
M. bovis sonicate	42*	50	64	42	58	47	60
Cytochrome C	95	100	80	70	100	1	1
Myoglobulin	85	86	100	69	92	5	3
Thyroglobulin	72	86	70	35	80	0	0
Transferrin	45	76	2	1	29	0	0
Fetuin	1	44	0	0	2	0	0
Egg albumin	64	44	5	29	85	0	0
Bovine albumin	64	77	100	34	82	0	0
DNA	3	26	0	0	24	0	0
Myosin	13	13	3	2	45	0	3
Rabbit actin	88	100	100	50	97	0	2
Bovine actin	87	95	87	26	92	0	0
Collagen	2	2	0	0	0	0	0

* Percentage of antibody binding to antigen compared to binding to a high control of mouse reference serum containing large amounts of IgM, IgA and IgG (Miles Laboratories Ltd., UK)

Table 3. Binding patterns of MoAb to certain tissue antigens

	MoAb				
Antigens	MB2	MB3	MB7	MB11	MB13
Transferrin	+*	+	−	−	+
Fetuin	−	+	−	−	−
Egg albumin	+	+	−	+	+
DNA	−	+	−	−	+

* Greater than 5% binding.

Table 4. Binding of polyclonal mouse antibody to *M. bovis* and tissue antigens

	% binding of antibody from BALB/c mice*	
Antigens	Sensitized with *M. bovis* strain 117	Unsensitized
M. bovis sonicate	100	30
Cytochrome C	40	32
Myoglobulin	47	36
Thyroglobulin	27	23
Transferrin	34	10
Fetuin	20	10
Egg albumin	53	12
Bovine albumin	34	30
DNA	53	8
Myosin	15	6
Rabbit actin	48	43
Bovine actin	37	33
Collagen	4	2

* Pooled serum from five mice in each group diluted 1 in 300.

Antigen competition

Table 5 shows the concentrations of actin, collagen and *M. bovis* strain AN5 sonicate needed to inhibit the binding of the MoAb by at least 50%. MoAb MB17 and MB5 were inhibited only by *M. bovis* sonicate (50 μg/ml). MoAb MB2, 3, 7, 11 and 13 were all inhibited to some extent by both *M. bovis* sonicate and actin. MoAb MB11 and 13 were inhibited by collagen at concentrations of 100 and 200 μg/ml, respectively, but the other MoAb were not inhibited by any concentrations of collagen used (maximum 200 μg/ml).

Table 5. The effect of actin, collagen and *M. bovis* sonicate on the binding of MoAb to their homologous antigen

	Concentration (μg/ml) of competitor needed to inhibit binding of MoAb by 50%		
MoAb*	Rabbit actin	Collagen	*M. bovis* strain AN5
MB2	200	>200	200
MB3	50	>200	12·5
MB5	>200	>200	50
MB7	200	>200	100
MB11	50	100	25
MB13	25	200	50
MB17	>200	>200	50

* MoAb used at a concentration that gives 75% binding to their homologous antigen.

Binding of MoAb to tissue sections

MoAb MB3, 5 and 17 were used. MB5 and MB17 failed to stain any of the sections of mesenteric lymph nodes from infected or non-infected guinea pigs or ferrets. MB3 bound strongly to all sections examined especially cytoplasm and membranes of the adipose tissue cells surrounding the mesenteric lymph nodes and the cells of the lymp node capsule. However, there was no staining where there were lesions rich with acid fast bacilli. Preincubation of MB3 with 5 μg of *M. bovis* sonicate overnight at 4°C inhibited the staining of tissue sections whereas 40 μg of an *E. coli* surface antigen extract failed to inhibit staining.

DISCUSSION

The MoAb examined bind to the *M. bovis* sonicate prepared from organisms grown on non-antigenic medium, demonstrating they are directed to mycobacterial epitopes. Our results show that five of the MoAb that bind to common epitopes of mycobacteria also bind to a range of tissue antigens. Nevertheless their binding patterns suggest that each recognizes different epitopes. Collagen was the only antigen examined where no binding occurred but in the antigen competition assays MoAb MB11 and MB13 were each inhibited by high concentrations of collagen. This anomolous result may be due to impurities since the collagen used in these experiments has been shown to contain three bands when tested to SDS-PAGE (Guilbert, Dighiero & Avrameas, 1982).

MoAb MB17 did not bind to any of the tissue antigens examined. This antibody binds to an epitope found on the surface of a wide range of *Mycobacterium* species (Morris *et al.*, 1985). MB5 appears to react only with *M. bovis* strains (Morris *et al.*, 1985) but in common with MoAb MB17 it did not bind to any of the tissue antigens examined. This result was confirmed by the antigen competition assays where the binding of MB5 was inhibited only by the homologous *M. bovis* antigen.

With the exception of MB5, the MoAb examined bind to a wide range of mycobacteria and of these all except MB17 bind to host tissue antigens. Mycobacterial antigens have been described that are common to all mycobacteria, most nocardiae and some corynebacteria and related genera (Stanford & Wong, 1975; Stanford *et al.*, 1975). The present study suggests that some of these common antigens may share epitopes with host tissue antigens. MoAb directed against shared epitopes should prove useful in isolating and identifying common antigens which up to now have not been specifically identified.

The significance of these cross-reacting epitopes is unclear. The epitope on mycobacteria recognized by MB3 is present in tissue from both infected and uninfected animals. Preliminary studies have indicated that antibodies with similar specificities as the MoAb that cross-react with host tissue antigens are present in sera from a range of apparently healthy animals (unpublished data). This finding may have important implications in the serodiagnosis of mycobacterial infections.

Recently Avrameas *et al.* (1983) have produced evidence for the existence of natural antibodies in normal individuals. Their specificities are very similar to some MoAb produced against *M. bovis* sonicates used in this study. They postulated that any increase in homologous antigens from the host or environment could produce an immunopathological state due, in part, to an increased production of high affinity natural antibodies. This view has more recently been endorsed by Grabar (1983).

Autoantibodies such as anti-nuclear and rheumatoid factors have been detected in patients with chronic tuberculosis (Lindquist, Coleman & Osterland, 1970) and our study has demonstrated that a number of mycobacterial antigens share epitopes with host tissue antigens. The relationship between these observations remains to be determined but the possibility that mycobacterial antigens may stimulate autoreactive clones is worthy of consideration since this may be significant in the development of the tubercle lesion and the pathogenesis of chronic tuberculosis.

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